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Pyrolysis-Mass Spectrometry/Pattern Recognition on a Well-Characterized Suite of Humic Samples

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Pyrolysis-mass spectrometry/pattern recognition on a well-characterized suite of humic samples

P. MACCARTHY, S. J. DELUCA and K. J. VOORHEES
Department of Chemistry and Geochemistry, Colorado School of Mines, Golden, Colorado 80401

and

R. L. MALCOLM and E. M. THURMAN
U.S. Geological Survey, Box 25046, Denver Federal Center, Denver, Colorado 80225

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Abstract—A suite of well-characterized humic and fulvic acids of freshwater, soil and plant origin was subjected to pyrolysis-mass spectrometry and the resulting data were analyzed by pattern recognition and factor analysis. A factor analysis plot of the data shows that the humic acids and fulvic acids can be segregated into two distinct classes. Carbohydrate and phenolic components are more pronounced in the pyrolysis products of the fulvic acids, and saturated and unsaturated hydrocarbons contribute more to the humic acid pyrolysis products. A second factor analysis plot shows a separation which appears to be based primarily on whether the samples are of aquatic or soil origin.

INTRODUCTION

HUMIC SUBSTANCES consist of a complex mixture of organic molecules which result from the decomposition of plant and animal residues in the environment (STEVENSON, 1982). These substances are ubiquitous, being found in soils, sediments and waters. Historically, most of the research on humic substances has been performed with soil samples. However, in recent years there has been an escalating interest in humic substances found in fresh water and sea water. Attempts at separating humic substances into pure components, or even into well defined fractions, have met with little success. All fractions obtained still consist of complex mixtures. Humic substances are operationally classified on the basis of their aqueous solubility as a function of pH into three main fractions: (a) fulvic acid, which is soluble at all pH values, (b) humic acid, which is soluble in alkaline media but precipitates at low pH, and (c) humin, which is insoluble at all pH values. Although many other fractions have been defined in the literature (KONONOVA, 1966), this classification is found to be generally useful to researchers and has been almost universally adopted.

In general, fulvic acids have higher oxygen and correspondingly lower carbon contents than humic acids. This can be largely accounted for in the greater carboxyl contents of fulvic acids compared to humic acids. These higher carboxyl contents, in conjunction with the smaller average molecular weights of fulvic acids, account for their greater hydrophilic character, and solubility at all pH's. Despite the above differences, there are no universal, clearcut distinguishing characteristics between fulvic acids and humic acids; rather, there is a gradual merging of the properties of one class into those of the other. While it is generally possible to distinguish between humic and fulvic acids *isolated from the same source* by elemental content, functional group content, infrared spectroscopy, etc., it has not been possible to unambiguously categorize humic and fulvic acids extracted from a diversity of sources using these approaches.

The purpose of this research was to apply pyrolysis-mass spectrometry (Py-MS) to a suite of well characterized humic substances and to subject the resulting data to pattern recognition and factor analysis in an attempt to see if this technique could discern the differences in source (river, soil, plant) and chemical nature (*e.g.*, carboxyl, phenol and carbohydrate contents, average molecular weight) of a variety of humic samples. Many papers have been published relating to the pyrolysis (WERSHAW and BOHNER, 1969) pyrolysis-gas chromatography (KIMBER and SEARLE, 1970; BRACEWELL and ROBERTSON, 1976) and pyrolysis-mass spectrometry (BRACEWELL, 1971; BRACEWELL and ROBERTSON, 1973; BRACEWELL *et al.*, 1975, 1980; MEUZELAAR *et al.*, 1977; DEHAAN, 1983; DEHAAN *et al.*, 1981) of humic substances. However, we are not aware of any prior research where Py-MS data collected from a wide variety of well characterized humic substances have been analyzed by pattern recognition and factor analysis. The suite of samples used in this study consisted of humic and fulvic acids from a wide range of soil and aquatic sources throughout the United States. In addition to the humic substances, a number of plant extracts and an organic sample of algal origin were included in the experiments.

EXPERIMENTAL

Humic substance samples

The humic substances, and other materials studied in these investigations, are described in Table 1, along with selected elemental, functional group and molecular weight data. All of the aquatic samples were isolated using the XAD-8 resin adsorption procedure (THURMAN and MALCOLM 1981) and the soil samples were extracted with dilute sodium hydroxide

Table I. SELECTED ANALYTICAL DATA RELATING TO ORGANIC MATTER SAMPLES INVESTIGATED

Sample and Sample No.	Elemental Content (Wt%) C	H	O	N	M.W.*	Carboxyl (meq/g)	Carbo-** hydrate (wt%)	Phenol (meq/g)
Missouri River								
(1,20) fulvic	54.5	5.1	35.9	1.0	800	6.0	-	0.1
(2,21) humic	50.6	4.86	-	2.43	-	5.0	-	0.8
Ohio River								
(3) fulvic	51.9	5.3	38.4	1.3	-	5.9	-	0.8
(4) humic	56.9	4.4	33.9	2.2	-	4.2	-	0.9
Ogeechee River								
(5) fulvic	51.7	5.2	40.5	0.9	-	5.4	-	1.0
(6) humic	55.0	4.8	27.1	1.3	-	2.8	-	1.5
Bear River								
(7) fulvic	54.8	4.4	38.4	1.2	-	6.2	-	0.8
(8) humic	55.0	-	-	-	-	4.1	-	2.0
Suwannee River								
(9) fulvic	51.3	4.32	42.9	0.6	1200	6.0	1.0	1.5
(10) humic	51.2	4.38	40.9	0.6	7300	4.7	-	1.8
North Carolina Podzol								
(11) fulvic	51.7	3.53	41.2	1.1	-	-	-	-
(12) humic	57.6	3.53	35.07	2.1	-	-	-	-
Colorado Mollisol								
(13) fulvic	-	-	-	-	-	-	10-15	-
(14) Willow extract	52.4	4.36	40.8	0.32	1200	3.77	16.4	3.57
Seward, Alaska, Spodosol								
(15) fulvic	-	-	-	-	-	-	-	-
(16) humic	-	-	-	-	-	-	-	-
(17) Algal	46.1	5.1	34.6	1.6	-	6.2	-	-
Maine Bog Water								
(18) fulvic	52.6	3.5	41.1	0.6	-	5.2	-	1.9
(19) humic	50.1	4.2	-	0.8	-	-	-	-
(22) Carex	52.1	5.08	37.1	1.43	2500	2.62	20.8	0.9
(23) Peat extract	52.1	4.65	36.7	1.6	2600	3.05	5.2	1.0
(24) Colorado bog water	52.6	4.79	36.7	0.95	2500-6000	4.9	2.5	1.4

*M.W.--molecular weight estimated from X-ray scattering data (Thurman et al., 1982).
**Glucose equivalent based on phenol-sulfuric acid method (Handa, 1966).

solution (MALCOLM 1976). All samples were hydrogen-exchanged by passage through a strong-acid cation exchanger in the hydrogen form and were then freeze-dried. The aquatic samples came from several sources: Missouri River (Iowa), Ohio River (Ohic), Ogeechee River (Georgia), Bear River (Colorado), Suwannee River (Georgia), Algal Lake (Nebraska), and Bogs (Maine and Colorado). The plant and peat samples were water extracts of air-dried materials and consisted of carex (sedge), willow and peat extracts. The soil samples comprised a North Carolina podzol, a mollisol from Colorado, and a spodosol from Alaska.

Equipment and procedures

Portions of the freeze-dried humic substances were dissolved or suspended (depending upon the solubility of the substance) in methanol at an approximate concentration of 10 mg/mL. The solutions/suspensions were then applied dropwise to rotating Curie-point wires, and the solvent was evaporated with a hot air gun (MEUZELAAR *et al.*, 1982). 510°C Curie-point wires were used and all samples were run in triplicate except for No. 22 which was run in duplicate.

The Py-MS analysis was carried out on the pyrolysis mass spectrometer system illustrated in Fig. 1. This system is comprised of a Fischer Curie-Point pyrolyzer (1.5 KW, 1.1 MHz power supply), an Extranuclear SpectrEL quadrupole mass spectrometer, and a Digital Equipment Corporation PDP 11/23 computer for instrument control and data collection. Low energy electron ionization (14eV) was used to reduce fragmentation (MEUZELAAR *et al.*, 1982). Ions in the mass range 10–240 amu were scanned at a rate of 1800 amu/sec.

Data analysis

Pyrolysis-mass spectrometry of humic substances generates a vast amount of data for each sample. Pattern recognition provides a tool for searching out similarities or differences between the samples based on mathematical analysis of these data.

The ARTHUR program (KOWALSKI and BENDER, 1972, 1973) was utilized to carry out pattern recognition of the Py-MS data. The raw mass spectral patterns were each normalized to their total ion counts before being input to ARTHUR. Sample spectra are shown in Fig. 2. Subroutines KAPRIN and KATRAN were used to calculate the principal components (*i.e.*, factors) of the correlation matrix. These factors form the basis of an orthonormal n-dimensional space in which each spectrum is represented by a point. This space is abstract; however, the factors are linear combinations of (scaled) mass spectral peak intensities. Graphical rotation of the factors (WINDIG *et al.*, 1981, 1983) can yield separations between properties or components of the sample set.

The factor analysis results were visualized by plotting each spectrum as a point in a two-dimensional factor space projection. These projections, called Karhunen-Loeve (K-L) plots (KOWALSKI, 1974), represent the factor scores of each sample

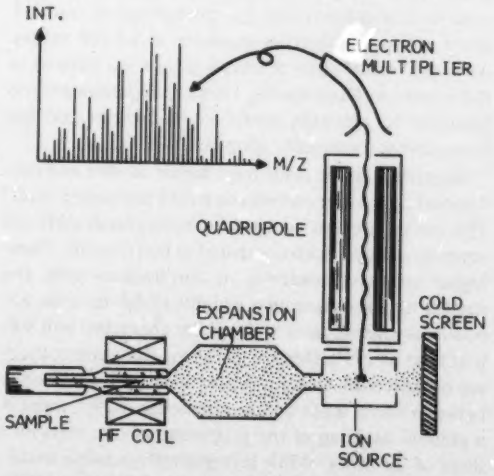

FIG. 1. Schematic Diagram of Pyrolysis-Mass Spectrometer System.

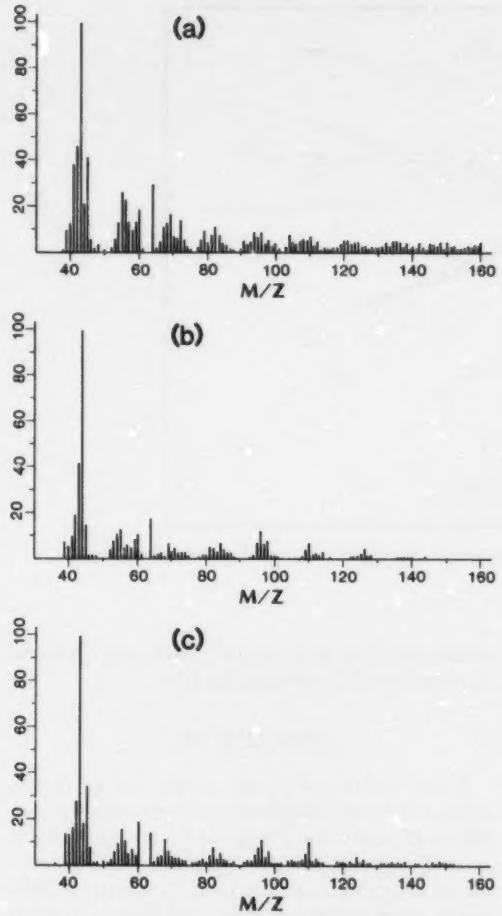

FIG. 2. Representative Normalized Mass Spectra of Pyrolysis Products: (a) Missouri River humic acid (Sample No. 2); (b) Colorado mollisol fulvic acid (Sample No. 13); (c) Alaska spodosol fulvic acid (Sample No. 15).

on each factor. The factor loadings, *i.e.*, the linear combination of peaks that make up a factor, are visualized in the factor spectra (VOORHEES and DURFEE, 1983, TSAO and VOORHEES, 1984, WINDIG *et al.*, 1981). Samples with high factor scores are enriched in components represented by positive peaks on the factor spectrum. The set of elemental and functional group data in Table I was also subjected to this type of factor analysis.

The method of nonlinear mapping was used to represent the entire n-dimensional space in two dimensions (KRUSKAL, 1964; SAMMON, 1968). Nonlinear maps for the pyrolysis products of the humic substances were produced in ARTHUR through subroutines DISTAN and NLM. The orthogonalized data produced in KAPRIN and KATRAN were input to DISTAN, which calculated the Euclidean distance from each point to every other point in the sample set. The distance matrix was then input to NLM which calculated two-dimensional coordinates of all the samples, preserving as much as possible the distances between each sample.

RESULTS AND DISCUSSION

Figure 3 shows the nonlinear map generated from the pyrolysis data for the 24 samples listed in Table I. The triangles represent triplicate determinations, and demonstrate satisfactory precision for the procedure. Samples 1 and 20 were "blind" duplicates and, as shown, the respective triangles fall close to each other on the nonlinear map. Similarly, samples 2 and 21, which were also identical, fall close to each other. It is possible to discern some of the separation patterns on the nonlinear map, for example, most (Nos. 11, 12, 15, 16) of the soil samples fall on the left-hand side of the plot.

The results of the factor analysis are shown in the form of K-L plots in Figs. 4 and 5. Again, the triangles in these plots indicate satisfactory precision for the replicate measurements. In Fig. 4, where factor 4 is plotted *versus* factor 1, the organic matter samples can be separated into two main groups, segregated by the dotted line. Interestingly, all of the fulvic acid samples lie above this line and all of the humic acid samples fall below it. The plant extracts (Nos. 14 and 22) fall with the fulvic acids as expected; these extracts resemble fulvic acid in molecular weight (800–1500) and are similar in water solubility. The bog fulvic acid (No. 18) and the bog water (No. 24) both lie in the fulvic acid region of the map but close to the dividing line. Evidently, this technique separates the humic acids from the fulvic acids on the basis of the pyrolysis-mass spectral data.

One might argue that the Py-MS/Pattern Recognition technique used here is accomplishing no more than merely illustrating the fact that these samples have already been separated into humic and fulvic acids prior to their analysis. However, such a criticism is not valid. Infrared or UV-visible spectroscopy cannot be used to unambiguously resolve the samples into humic and fulvic acid categories. Furthermore, the simple solubility criterion does not always work; for example, freeze-dried aquatic *humic* acids frequently dissolve readily in water. An examination of the analytical data in Table I shows considerable overlap between the characteristics of the humic acids and fulvic acids. In fact, when the data in Table I were subjected to the identical type of factor analysis/pattern recognition to which the pyrolysis data were subjected, no separation, analogous to that in Fig. 4, could be discerned! In order to establish what structural features of the substances may be responsible for this segregation (Fig. 4) one must examine the factor spectra. Figure 6 shows the factor spectrum generated by rotating 10° normal to the factor 1–4 plane (this corresponds to a transformation of factor 1 into the dotted line shown in Fig. 4). The major features causing the separation between humic acid and fulvic acid pyrolysis products seem to be carbohydrates, phenols, and hydrocarbons. The series of carbohydrate peaks (m/z = 44, 68, 96, 98, 110, 126) in the positive direction (Fig. 6) is representative of the pyrolysis products of the fulvic acids. There is also a phenolic component (m/z = 94, 122, 150) contributing to the fulvic side of the fulvic acid/humic acid separation. The humic acid side of the spectrum (negative direction) is dominated by saturated (m/z

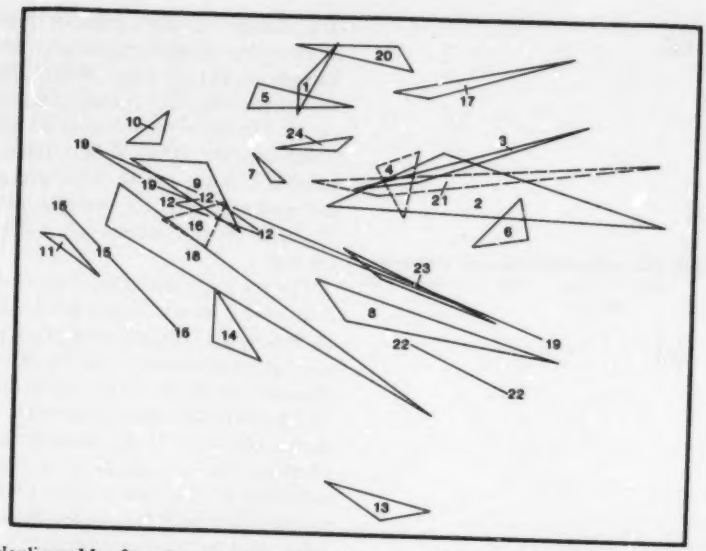

FIG. 3. Nonlinear Map from Pyrolysis-Mass Spectral Data on 24 Samples in Table I. Numbers correspond to sample numbers in Table I.

= 72, 100) and unsaturated (m/z = 42, 56, 70, 112) hydrocarbon peaks.

Figure 5 shows the K-L plot with factor 2 plotted as a function of factor 1. In this case the organic matter samples are found to separate into two groups. This separation results primarily from the pyrolysis products CO_2 (m/z = 44) and SO_2 (m/z = 64), and appears to be related to the sample sources in that samples from aquatic environments are distributed mainly in the upper right region of the diagram and soil-derived samples dominate the lower left portion of the diagram; humic and fulvic acids from a given source always lie on the same side of the dividing line.

CONCLUSIONS

Factor analysis of Py-MS patterns of 24 diverse humic substances and plant extracts has been successfully employed to discriminate humic acids from fulvic acids. Components of the pyrolysate which account for the discrimination have been identified; carbohydrates and phenolics are richer in the fulvic acid pyrol-

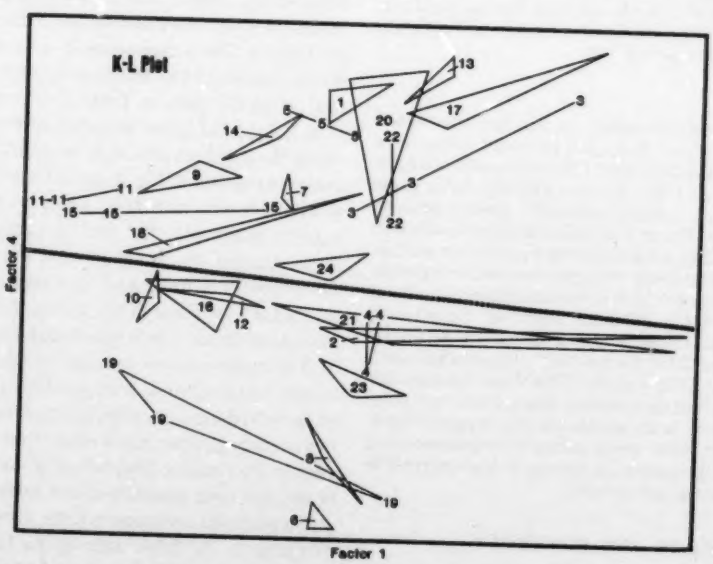

FIG. 4. K-L Plot of Pyrolysis-Mass Spectral Data with Factor 4 plotted versus Factor 1. Numbers correspond to sample numbers in Table I.

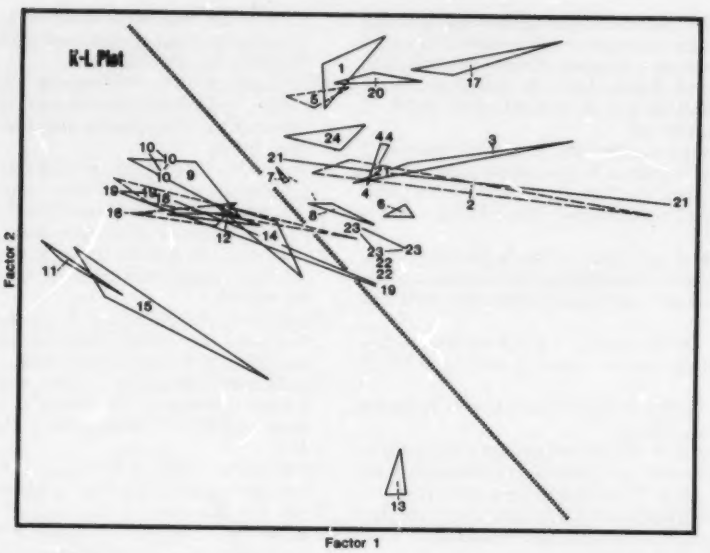

FIG. 5. K-L Plot of Pyrolysis-Mass Spectral Data with Factor 2 plotted *versus* Factor 1. Numbers correspond to sample numbers in Table I.

ysates while saturated and unsaturated hydrocarbon fragments contribute more to the humic acid pyrolysates. A second separation was also observed which appears to differentiate between aquatic and soil-derived humic substances.

In considering the implications of these results it should be remembered that factor analysis is simply a method of visualizing a multidimensional data space. In this case, the data space consists of mass spectra of pyrolysis products. The 24 samples analyzed in this study were found to be distributed (Fig. 4) into two discrete regions (fulvic acids and humic acids) in the data space, indicating that the chemical differences between the humic and fulvic acids, which are responsible for their operational separation, are reflected in the Py-MS patterns. The second separation (Fig. 5) suggests a fundamental difference between aquatic and soil-derived humic substances. The Py-MS/Pattern Recognition technique discussed here appears to be a useful, if not unique, tool for discriminating between humic and fulvic acids, and may also be useful for identifying the sources of these materials.

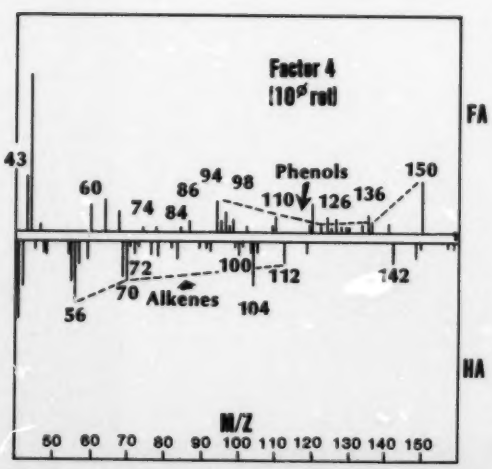

FIG. 6. Factor Spectrum of Factor 4 (rotated 10° in the 1, 4 plane) Generated from Pyrolysis Data on 24 Samples in Table I.

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